

DP Barcode : D180148
PC Code No : 109701
EEB Out

DEC 20 1993

To: Linda DeLuise
Product Manager 52
Special Review and Reregistration Division (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 109701
Chemical Name : Permethrin
Type Product : Insecticide - synthetic pyrethroid
Product Name : Ambush, Pounce
Company Name : FMC Corp and ICI Americas Inc.
Purpose : Review daphnia chronic study

Action Code : 627 Date Due : ~~08/20/92~~ 12/31/93
Reviewer : Renee Lamb

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)	413157-01	N	124-2		
72-1(B)			72-4 (B)	47039		141-1		
72-1(C)			72-6			141-2		
72-1(C)						141-5		

A=Acceptable (Study satisfied Guideline)/Concur

P=Partial (Study partially fulfilled Guideline but additional information is needed)

S=Supplemental (Study provided useful information but Guideline was not satisfied)

N=Unacceptable (Study was rejected)/Nonconcur

12/20/93



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 21 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: Data review for Permethrin (109701)

From: *for* Anthony F. Maciorowski, Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) 12/20/93

To: Linda DeLuise
Product Manager 52
Special Review and Reregistration (7508W)

EEB has completed the review of the data submission for permethrin. The following is a brief summary of the data reviewed:

CITATION: Forbis, A.D. and W.A. McAllister. 1980. Chronic Toxicity of ¹⁴C-Permethrin to *Daphnia magna* Under Flow-Through Test Conditions. ABC Report No. 23647. Study conducted by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by ICI Americas, Inc., Goldsboro, NC. EPA MRID No. 47039.

CONCLUSIONS: This study is not scientifically sound and does not meet the guideline requirements for a daphnid life-cycle test.

Mean measured concentrations were highly variable during the test. The measured concentrations were 121-142% of the nominal concentrations. Guidance from the September 1992 draft document from EFED to the Ecological Risk Assessment Work Group (ERAWG)/National Agricultural Chemicals Association (NACA) discussing acceptable aquatic lab studies intimates that, "An increase in measured test concentration of more than 30% from the nominal concentration during the test will generally not result in rejection, provided that the following conditions are met:

1. a reasonable and scientific explanation is given, and the variability of results produced by the chemical analysis method is adequately characterized;
2. all test containers exhibit a similar (but not necessarily identical) shift;
3. the variability of the measured concentrations is acceptable;



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4. a statistically valid endpoint can be derived from the measured concentrations, and

5. the preliminary stability information is provided with complete documentation and description of methods used to derive such information."

A stability study¹ (MRID No. 408703-01) was submitted along with the daphnia study. This study reported that the "stability of ¹⁴C-permethrin in acetone and in aqueous solution as observed in this study gives credibility to the conclusion that the stock solution employed in the 21-day chronic study was stable under the conditions employed for that study as described in the aforementioned report." However, the results from this study do not explain the high degree of variability observed in the daphnia chronic study. No explanation of these variations was reported by the author. This variation may be an indication of diluter malfunctions or an error in stock solution preparation.

In addition, daphnid growth was not measured during the test. Data on survival and growth must be reported as stated in the ASTM guidelines "Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna" (E 1193-87) as either length or dry weight .

Also, water quality was monitored during the first half of the study only. As stated in the aforementioned guidelines, E 1193-87, "Hardness, alkalinity, conductivity, and pH should be measured...at least weekly during the test..."

If there are any questions, contact Renee Lamb at 305-5294.

¹ This study was not reviewed by EEB and does not need to be sent to EFGWB for review.

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DATA EVALUATION RECORD

1. **CHEMICAL:** Permethrin. Shaughnessey No. 109701.
2. **TEST MATERIAL:** ^{14}C -Permethrin; 94.8% purity; dissolved in hexane.
3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Flow-Through Test. Species Tested: *Daphnia magna*.
4. **CITATION:** Forbis, A.D. and W.A. McAllister. 1980. Chronic Toxicity of ^{14}C -Permethrin to *Daphnia magna* Under Flow-Through Test Conditions. ABC Report No. 23647. Study conducted by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by ICI Americas, Inc., Goldsboro, NC. EPA MRID No. 47039.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Rosemary Graham Mora*Date: *13 Sept 93*
*11/16/93*6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *P. Kosalwat*Date: *11/13/93*

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: *Henry T. Craven*Date: *11/16/93*

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a daphnid life-cycle test. Mean measured concentrations were highly variable during the test and water quality was monitored during the first half of the study only. In addition, daphnid growth was not measured during the test. ~~The mean of ^{14}C -permethrin for *Daphnia magna* was $>6\mu\text{g/L}$ and $<118\text{ ng/L}$ mean measured concentrations (geometric mean 84.1 ng/L).~~ *11/16/93*

8. **RECOMMENDATIONS:**9. **BACKGROUND:**10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

- A. **Test Animals:** First instar *Daphnia magna* were obtained from an in-house culture. The culture was maintained at $20 \pm 2^\circ\text{C}$ in ABC well water. The photoperiod was 16 hours of light. Cultured daphnids were fed a suspension of "PR-11" and yeast *ad libitum*.
- B. **Test System:** The test was conducted under flow-through conditions using a 1-l intermittent proportional diluter. Test vessels were 1-l glass beakers each with a notched drain covered with 50-mesh Nitex® screen. The diluter delivered water to each vessel at an average rate of 250 ml every 45 minutes which provided 8 volume replacements daily. The diluter was allowed to equilibrate for approximately 24 hours prior to test initiation. Test temperature was maintained at $21 \pm 2^\circ\text{C}$ by a temperature-controlled water bath.

The dilution water was aerated ABC well water with a pH of 8.2, a conductivity of 50 $\mu\text{mhos/cm}$, and a hardness and alkalinity of 255 and 368 mg/l as CaCO_3 , respectively.

A primary stock solution was prepared using the radiolabelled test material and nanograde acetone. Secondary stock solutions were prepared in acetone throughout the test period.

- C. **Dosage:** Twenty-one-day, flow-through test. Five nominal concentrations (21, 45, 97, 190, and 470 ng/l) were selected for this study. A dilution water control and a solvent control were also included. The solvent control had an acetone concentration of 0.57 ml/l which was equivalent to the solvent concentration in the highest test level.
- D. **Design:** Fifteen first-instar daphnids (<24 hours old) were randomly selected and placed in three of four replicate chambers (i.e., 45 daphnids/treatment). The remaining replicate chamber was used on a rotating basis when cleaning was necessary. The test vessels were cleaned at least twice weekly. The daphnids were fed a liquid food suspension twice daily.

Adult survival was assessed on day 7 at which time the number of test organisms was randomly reduced to 10 per chamber. Reproduction was recorded on exposure days 14 and 21 at which time the young were counted and

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discarded. Day 7 was assumed to be the first day of reproduction.

Dissolved oxygen concentration (DO), temperature, and pH were measured on days 0, 3, and 9 in replicates of the high and low test concentrations and the dilution water control. Temperature was also monitored continuously in one control chamber.

On test days 0, 3, 7, 10, 14, and 21, water samples were collected from each treatment for determination of ¹⁴C-permethrin. Analysis was performed using liquid scintillation counting.

- E. **Statistics:** Measured biological parameters were analyzed using a one-way analysis of variance (ANOVA) along with Fisher's Protected Least Significant Difference as necessary. Control and solvent control data were analyzed using the ANOVA; if no significant difference was determined between the two then solvent control data were used for treatment comparison. All conclusions were based on a 95% confidence level.

12. **REPORTED RESULTS:** Mean measured concentrations were 29, 60, 118, 271, and 608 ng/l which represent 121-142% of nominal concentrations (Table 2, attached).

Survival was significantly reduced at 271 ng/l after 7 days of exposure (Table 4, attached). Total mortality was noted in the highest test concentration by day 7. No other significant reduction in survival occurred. Mean number of young per reproductive day was significantly reduced at 271 ng/l by day 14 and at 118 and 271 ng/l by day 21 (Table 4, attached).

Based on measurements conducted on days 0, 3, and 9, the test solutions had a pH of 8.0-8.2, a DO of 8.0-9.3 mg/l, and a temperature of 21°C. "Temperature was monitored continuously for the duration of the study in the control and varied only 3°C...Water chemistry should have been performed on at least day 21 but was not. However, reproduction and survival of the two controls give good evidence that the water quality parameters were acceptable for the study."

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
 "Based on the data from this 21 day *Daphnia magna* dynamic life cycle bioassay the MATC limits are estimated to be between the mean measured ¹⁴C-permethrin concentrations of 60 ng/l and 118 ng/l."

A GLP compliance statement was included in the report indicating that this study was conducted following the intent of FDA's Good Laboratory Practice Regulations (21 CFR Part 58). A Quality Assurance statement was also included.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Test Procedure:** An SEP for *Daphnia* chronic flow-through studies is not available at this time, thus the SEP for static-renewal studies was used as a general guidance in this data validation process.

Daphnid growth was not measured during this study.

On day 7, the number of test organisms in each replicate was reduced from 15 to 10. No justification was given for this reduction.

Temperature, pH, and DO in the control, low, and high test solutions were measured only on days 0, 3, and 9. The DO should be measured weekly in all treatments. Hardness, alkalinity, pH, and conductivity should be measured weekly in the control and one concentration.

The photoperiod used during the test was not reported.

No batch or lot number of the test material was reported.

The highest acetone concentration (0.57 ml/l) used in this study exceeded the recommended concentration for a flow-through study (0.1 ml/l).

Measured concentrations during the test were highly variable. The highest measured values ranged from 132 to 210% of the lowest measured values of the same nominal concentrations (Table 2, attached).

- B. Statistical Analysis:** The reviewer used the computer program Toxstat® to analyze reproduction data. The homogeneity of variance and normality of all data were examined using Hartley's test and the chi-square test, respectively. The number of young per reproductive day (square-root transformed) met the assumptions of homogeneity of variance and normality, therefore, this parameter was analyzed using William's test (printout, attached). The reviewer's result for the number of young per reproductive day was the same as the authors'. Visual examination of the survival data was adequate to determine significant reductions to

survival. The reviewer's conclusion agrees with the authors'.

- C. Discussion/Results: This study is not scientifically sound and does not meet the guideline requirements. Measured concentrations were highly variable during the test and water quality was monitored until day 9 only. In addition, daphnid growth was not measured during the study. ~~The MATC of ¹⁴C-permethrin for *Daphnia magna* was between 60 and 118 ng/l mean measured concentrations. The geometric mean of the MATC was 84.1 ng/l.~~

D. Adequacy of the Study:

- (1) Classification: Invalid.
- (2) Rationale: 1) Measured concentrations were highly variable; 2) Water quality was monitored only during the first half of the study; 3) Daphnid growth was not measured during the study.
- (3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes; 27 August 1993.

Page _____ is not included in this copy.

Pages 9 through 11 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
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NUMBER OF GROUPS: 7

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Permethrin: No. of Young/Reproduction Day
File: 47039.rep Transform: SQUARE ROOT(Y)

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
	<hr/>	<hr/>	<hr/>	<hr/>	<hr/>
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	8	6	7	0

Calculated Chi-Square goodness of fit test statistic = 5.7230

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Permethrin: No. of Young/Reproduction Day
File: 47039.rep Transform: SQUARE ROOT(Y)

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 534.07
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Permethrin: No. of Young/Reproduction Day
 File: 47039.rep Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	3	3.667	1.913	1.956
2	Solvent Control	3	3.833	1.956	1.956
3	29 ng/l	3	4.000	1.999	1.956
4	60 ng/l	3	3.633	1.904	1.904
5	118 ng/l	3	2.967	1.720	1.720
6	271 ng/l	3	0.900	0.944	0.944
7	608 ng/l	3	0.000	0.004	0.004

Permethrin: No. of Young/Reproduction Day
 File: 47039.rep Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	1.956				
Solvent Control	1.956	0.525		1.76	k= 1, v=14
29 ng/l	1.956	0.525		1.85	k= 2, v=14
60 ng/l	1.904	0.113		1.88	k= 3, v=14
118 ng/l	1.720	2.360	*	1.89	k= 4, v=14
271 ng/l	0.944	11.839	*	1.90	k= 5, v=14
608 ng/l	0.004	23.317	*	1.91	k= 6, v=14

s = 0.100

Note: df used for table values are approximate when v > 20.